EXHIBIT 16

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Congenital malformations due to antiepileptic drugs

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Abstract

To identify the major risk factors for the increased incidence of congenital malformations in offspring of mothers being treated for epilepsy with antiepileptic drugs (AEDs) during pregnancy and, to determine the relative teratogenic risk of AEDs, we prospectively analyzed 983 offspring born in Japan, Italy, and Canada. The incidence of congenital malformations in offspring without drug exposure was 3.1%, versus an incidence with drug exposure of 9.0%. The highest incidence in offspring exposed to a single AED occurred with primidone (PRM; 14.3%), which was followed by valproate (VPA; 11.1%), phenytoin (PHT; 9.1%), carbamazepine (CBZ; 5.7%), and phenobarbital (PB; 5.1%). The VPA dose and level positively correlated with the incidence of malformations. This study first determined a cut-off value of VPA dose and level at 1000 mg/day and 70 μg/ml, respectively, to avoid the occurrence of malformations. The incidence of malformations increases as the number of drugs increases, and as the total daily dose increases. Specific combinations of AEDs such as VPA + CBZ and PHT + PRM + PB produced a higher incidence of congenital malformations. The incidence of malformations was not associated with any background factors studied except for the presence of malformations in siblings. These results indicate that the increased incidence of congenital malformations was caused primarily by AEDs, suggesting that malformations can be prevented by improvements in drug regimen, and by avoiding polypharmacy and high levels of VPA (more than 70 μg/ml) in the treatment of epileptic women of childbearimg age.

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1. Introduction

Improved diagnosis and therapy of epilepsy has allowed most women with epilepsy to marry and bear children. However, the incidence of congenital malformations in offspring of mothers with epilepsy who were treated with antiepileptic drugs (AEDs) during pregnancy is higher than the normal population (Dansky et al., 1982; Nakane, 1982) and higher than in offspring whose mothers were not treated with AEDs during pregnancy (Koch et al., 1982; Lindhout et al., 1982; Kaneko et al., 1986).

In recent prospective studies, teratogenicity appeared to be attributable to AEDs rather than to epilepsy (Kaneko et al., 1986, 1992). Folate deficiency in response to AED therapy (Ogawa et al., 1991; Dansky et al., 1992) and genetically determined drug detoxifying enzyme activity have been related to the occurrence of malformations (Buehler et al., 1990). The involvement of other risk factors, however, such as maternal hypoxia, and genetic influences, must also be considered in the etiology of congenital malformations. The aim of the present study was to identify the major risk factors for the occurrence of congenital malformations, the relative teratogenic risk of each AED, and the advisable ranges of AED doses to reduce the risk of congenital malformations in offspring.

2. Subjects and methods

For this prospective study, the same study design was used to collect and analyze the same data from three countries (Japan (Hirosaki, Fukushima, Nagoya, and Nagasaki), Italy (Milan), and Canada (Montreal)). This study was approved by the Ethics Committee in each center.

Recruitment of subjects began in April 1978, and was completed in December 1991. At each center, where the study was introduced, the nature and purpose of the study was explained to every female patient of childbearing age with epilepsy who visited the clinic. Those who consented were followed by a team of obstetricians and neurologists at a minimum of monthly intervals throughout their pregnancy. Most of our subjects have been studied from the first trimester of pregnancy, and a few subjects were studied before conception. The population of the study group was composed of women with different socio-cultural backgrounds, mainly from the suburban areas around each medical center.

A total of 1072 patients were enrolled (441 in Japan, 371 in Italy, and 260 in Canada). Of the 1072 patients 54 were excluded because they did not follow up as scheduled, usually due to change of address, so that their data were not complete. Cases of spontaneous (19) and elective abortion (16) were also excluded, so the study results are based upon the 983 pregnancies which were under treatment until term and for which the data were complete.

Blood samples were taken to measure AED levels, folate levels and for routine laboratory tests at each visit before the morning dose of AED had been taken. AED doses were kept constant throughout pregnancy, unless a significant worsening in seizure frequency occurred.

To obtain a precise record, congenital malformations were examined at each center at birth, at 5 days, and at the 1-month visit by a team of obstetricians and neurologists, according to a standardized check-list based on the report of the Japanese Association of Obstetricians for Maternal Welfare (Gomibuchi, 1977; Kaneko et al., 1988).

The following data were recorded: maternal age at delivery; gravida; gestational week; outcome of previous pregnancy; etiology of epilepsy; classification of epilepsy; classification of seizure; occurrence of seizure during the first trimester of pregnancy; change in seizure frequency during pregnancy. Drug score (DS) of 16 AEDs, and level score of five AEDs were recorded (Table 1). According to seizure frequency, patients were allocated into six groups: (1) seizure free; (2) less than one seizure/month; (3) one to three seizures/

Table 1
The level score, the drug score and the number of offspring exposed to each drug

AEDs	Level (μg/ml)[a]	Dose (mg/day)[b]	n
1 Phenytoin (PHT)	3.0	50	423
2 Phenobarbital (PB)	4.0	50	275
3 Primidone (PRM)	—	100	184
4 Carbamazepine (CBZ)	1.1	100	318
5 Valproate (VPA)	10.0	100	167
6 Ethosuximide (ETS)	8.0	250	24
7 Sulthiame	—	50	7
8 Acetazolame (AZA)	—	125	18
9 Ethylphenacemide (EPM)	—	200	6
10 Diazepam (DZP)	—	5	15
11 Nitrazepam (NZP)	—	5	5
12 Methylphenobarbital (MPB)	—	75	23
13 Acetylpheneturide (APT)	—	100	5
14 Zonisamide (ZNS)	—	75	3
15 Clonazepam (CZP)	—	1	21
16 Clobazam	—	5	1

Drug score or level score is the ammount of or level of each of the main drugs constituting 1 U in the calculation of total and maximum daily dose or level.
n, the number of offspring exposed to each AED.
[a] Level score.
[b] Drug score.

month; (4) one to five seizures/week; (5) daily seizures; and (6) more than two seizures/day. Seizure frequency during pregnancy was compared with the 9-month period before pregnancy. Passage from one frequency group to another was defined as an increase or decrease in seizure frequency (Canger et al., 1982).

The DS system (Table 1) (Kaneko et al., 1988) was adopted to detect any correlation between the effects of daily AED dosage and the occurrence of malformations. The DS system was prepared according to the clinical efficacy of AEDs (Richens and Rowe, 1970). This DS system was utilized because many of the subjects had been treated with polypharmacy, and because the teratogenic effects of AEDs might be different from drug to drug. The score represents the total dosage of AEDs administered each day during the first trimester of pregnancy. The drug-level scoring system expressed in units is as follows: 1 U = 4 μg/ml for phenobarbital (PB), 1.1 μg/ml for carbamazepine (CBZ), 3 μg/ml for phenytoin (PHT), 10 μg/ml for valproate (VPA), and 8 μg/ml for ethosuximide (ESM). Data of tobacco and alcohol consumption were not recorded mainly because of the unreliability of this historical information.

3. Statistics

For statistical analysis, first we estimated the incidence of malformations in the total group and in each subgroup. Next, we examined the correlation between the occurrence of malformations and the following factors: (a) background factors of the mothers, (b) AED combination profiles, (c) the number of AEDs administered, and (d) AED doses and levels.

We used the following tests for each data group: exact probability method, chi-square test, Wilcoxon rank sum test and Welch's test in evaluating (a), chi-square test and Fisher's exact probability test in (b), trend test for contingency table in (c), and Welch's test and logistic regression analysis in (d). Logistic regression analysis is a method that regress a logit transformed a binary response on several explanatory variables and explore influ-

ential explanatory variables on the response. We applied this method with the occurrence of malformation as response and dose of each AED as explanatory variable, and evaluated whether the dose-response relationship was significant or not.

Finally, for each AED administered, the minimum AED dose and drug level were determined that would significantly increase the incidence of malformations. The drug scores of the single-AED treated cases and multiple-AED treated cases were analyzed by the maximum chi-square test (Miller and Siegmund, 1982).

4. Results

Among 983 pregnancies, 83 malformed infants were observed (8.4%). The overall incidence of malformations in AED exposed offspring was 9.0% (80/885). Three anomalies such as a flexion of the thumb, distorted fingers, and a small nevus were observed in AED unexposed offspring. None of the mothers had any associated chronic illness.

4.1. Number of each malformation

The number and type of malformations were as follows: four spina bifida, three hydrocephalus, six facial with or without neck malformations, 14 heart malformations, two circulatory system malformations other than heart, 11 cleft lip and/or palate, 17 inguinal hernias, one esophagial atresia, 13 urogenital malformations, ten skeletal malformations, four umbilical hernias, one heterotaxia, one Down syndrome, one lung cyst, and one Smith-Lemli-Opitz syndrome.

4.2. Drug specific malformations

The relationship between type of malformation and type of AED was studied. AED exposure, by type and number of drugs, was correlated with the kinds of malformations seen. Of the cases treated with monopharmacy, inguinal hernias were observed in seven offspring, and three of the seven offspring had been exposed to primidone (PRM). Among all malformations, only inguinal hernia was significantly related to PRM exposure ($P = 0.032$, $n = 35$). The remaining malformations were not significantly related to any specific AED in the present subjects.

4.3. Maternal background factors and occurrence of malformations

Table 2 shows the relationships between the background factors of epileptic mothers and the occurrence of malformations. Among the factors analyzed, only the presence of malformation in siblings correlated with the occurrence of malformations ($P = 0.011$). Although there was a trend for a higher incidence of malformations in patients with idiopathic epilepsy, the association failed to reach a statistically significant level ($P = 0.142$). Neither epileptic seizure type, type of epilepsy, occurrence of seizures during the first trimester of pregnancy, nor seizure frequency correlated with the malformations. Maternal factors were further analyzed by logistic regression model to see whether or not any relationships existed between the occurrence of malformation (response) and these factors (explanatory variable: stimulus). However, there was no significant relationship evident.

4.4. AED combination profiles and corresponding incidences of malformations

Of the 98 offspring unexposed to AED in utero, three had malformations (3.1%) as shown in Table 3. The overall incidence of malformations in all offspring exposed to six major AEDs was: 17.6% for methylphenobarbital (MPB), 13.0% for PRM, 12.0% for VPA, 10.4% for PHT, 9.8% for PB, and 6.6% for CBZ. The incidences of malformations in relation to the number of AEDs to which offspring were exposed were: 7.8% for one AED, 9.6% for two AEDs, 11.5% for three AEDs, 13.5% for four AEDs, and 15.4% for five AEDs. The incidence increased as the number of AEDs to which the fetus was exposed increased ($P = 0.012$).

Of the AED profiles with more than five cases, VPA + CBZ and PHT + PB + PRM showed the two highest rates of malformations of 21.4% (odds ratio: 8.6) and 24% (odds ratio: 10.0), re-

Table 2
Characteristics of epileptic mothers, and relationships between maternal factors and occurrence of malformations

Item	Category	Normal	Malformed	Total	Statistical test
Age at delivery	Mean	26.99	27.07	27.00	Welch's test, $P = 0.874$
	S.D.	4.44	4.44	4.44	
Gravida	1	440 (49.2)	35 (42.2)	475	$z = -1.275$
	2	247 (27.6)	27 (32.5)	274	$P = 0.202$
	3	138 (15.4)	8 (9.6)	146	
	4	40 (4.5)	10 (12.0)	50	
	Over 5	30 (3.4)	3 (3.6)	33	
	Unknown	5	0	5	
Gestational age	Mean	39.39	39.25	39.38	Welch's test, $P = 0.554$
	S.D.	1.60	2.03	1.64	
Outcome of previous pregnancy	1	441 (49.3)	35 (43.2)	476	$\chi^2 = 6.765$
	2	74 (8.3)	10 (12.3)	84	df = 8
	3	97 (10.9)	5 (6.2)	102	$P = 0.562$
	4	243 (27.2)	29 (35.8)	272	
	5	18 (2.0)	1 (1.2)	19	
	6	4 (0.4)	0 (0.0)	4	
	7	10 (1.1)	1 (1.2)	11	
	8	3 (0.3)	0 (0.0)	3	
	9	4 (0.4)	0 (0.0)	4	
	Unknown	6	2	8	
Maternal malformation	None	688 (99.3)	73 (98.6)	761	$P = 0.457$
	Malformed	5 (0.7)	1 (1.4)	6	
	Not reported	207	9	216	
Paternal malformation	None	686 (99.0)	74 (100.0)	760	$P =$ near 1
	Malformed	7 (1.0)	0 (0.0)	7	
	Not reported	207	9	216	
Sibling malformation	None	498 (96.3)	52 (88.1)	550	$P = 0.011$
	Malformed	19 (3.7)	7 (11.9)	26	
	Not reported	383	24	407	
Etiology of epilepsy	Idiopathic	533 (66.2)	52 (75.4)	585	$P = 0.142$
	Symptomatic	274 (33.8)	17 (24.6)	291	
	Unknown or undifferentiated	93	14	107	
Seizure type	Generalized	385 (43.9)	39 (47.0)	424	$\chi^2 = 0.274$
	Partial	492 (56.1)	44 (53.0)	536	df = 1
	Unknown or undifferentiated	23	0	23	$P = 0.644$
Seizure during first trimester	None	446 (51.8)	42 (51.9)	488	$P =$ near 1
	Present	415 (48.2)	39 (48.1)	454	
	Unknown or uncertain report	39	2	41	
Seizure frequency during pregnancy	Decreased	57 (6.9)	7 (9.1)	64	$z = 1.489$
	No change	649 (78.2)	63 (81.8)	712	$P = 0.136$
	Increased	124 (14.9)	7 (9.1)	131	
	Unknown or uncertain report	70	6	76	

Table 2
Characteristics of epileptic mothers, and relationships between maternal factors and occurrence of malformations

Item	Category	Normal	Malformed	Total	Statistical test
Generalized convulsion (A), occurrence of seizure (B)	A: No, B: No	87 (10.1)	8 (9.9)	95	$\chi^2 = 1.043$
	A: No, B: Yes	105 (12.2)	7 (8.6)	112	df = 3
	A: Yes, B: No	356 (41.5)	34 (42.0)	390	$P = 0.791$
	A: Yes, B: Yes	310 (36.1)	32 (39.5)	342	
	Unknown or uncertain report	42	2	44	

z, Normal deviate; 1, primipara; 2, spontaneous abortion; 3, artificial abortion; 4, spontaneous delivery; 5, Cesarean section; 6, forceps delivery; 7, vacuum delivery; 8, breech presentation; 9, stillborn.

spectively. The analysis of all AED exposed offspring revealed that there were significant associations between occurrence of malformations and doses of PHT ($P = 0.015$), dose of VPA ($P = 0.026$), number of AEDs ($P = 0.018$), and the DS ($P = 0.0059$).

4.5. Relationship between AED related factors and occurrence of malformations

4.5.1. Analysis of 500 monopharmacy cases

The incidence of malformations in offspring exposed to a single AED (500 offspring) was as follows: PHT, 9.1%; PB, 5.1%; PRM, 14.3%; CBZ, 5.7%; VPA, 11.1% (Table 3). There was no significant difference between these percentages. However, PRM ($P = 0.029$) and VPA ($P = 0.039$) exposure showed significantly higher incidences of malformations than in offspring without drug exposure. The corresponding odds ratios were 5.3 and 4.0, respectively. VPA dose ($P = 0.004$) and the DS ($P = 0.040$) for mothers of malformed offspring were significantly higher than those of mothers of infants without malformation (Table 4).

The stimulus (AED dose)-response (occurrence of malformation) relationship was studied with logistic regression analysis. VPA dose showed a significant positive correlation with the occurrence of malformations (regression coefficient = 0.0021, $P = 0.0075$) (Fig. 1a), while the remaining AEDs did not. The DS did not correlate with malformations.

AED levels of 333 of 500 mothers treated with monopharmacy were determined during the first trimester of pregnancy. The mean VPA level of mothers of malformed offspring (77.80 ± 19.99 μg/ml, $n = 8$) was significantly higher than those with normal offspring (46.86 ± 21.22 μg/ml, $n = 51$) ($P = 0.023$). There was not such a significant correlation between any other AEDs and malformations. The total level score did not correlate with the occurrence of malformations. Logistic regression analysis revealed a significant association between VPA level and the occurrence of malformations (Fig. 1b) (regression coefficient = 0.052, $P = 0.005$). The remaining AED levels did not show such a significant association.

4.5.2. Analysis of 385 polypharmacy cases

The stimulus (AED dose)-response (occurrence of malformation) relationship was studied with logistic regression analysis, but no significant correlation was obtained. The DS significantly, positively correlated with malformations as shown in Fig. 2 (regression coefficient = 0.0505, $P = 0.0059$). Logistic regression analysis revealed a significant association between VPA level and the occurrence of malformations (regression coefficient = 0.035, $P = 0.001$). The remaining AED levels did not show such a significant association.

4.5.3. AED doses to which malformed offspring were exposed in utero

In all mothers of offspring with malformations, those who had been given PRM had received a dose of 400 mg or more, and those who had been

Table 3
AED combination profiles and incidences of malformations

Number of AED	PHT	PB	PRM	CBZ	VPA	MPB	Other AED	Malformed	Total	O.R.	Incidence (%)	
0								3	98		3.1	
1							○	0	10		0.0	7.8 (39/500)
						○		0	5		0.0	
					○			9	81	4.0	11.1	
				○				9	158	1.9	5.7	
			○					5	35	5.3	14.3	
		○						4	79	1.7	5.1	
	○							12	132	3.2	9.1	
2					○		○	0	3		0.0	9.6 (22/231)
				○			○	0	4		0.0	
				○	○			3	14	8.6	21.4	
			○				○	0	5		0.0	
			○		○			0	3		0.0	
			○	○				0	6		0.0	
		○					○	1	6	6.3	16.7	
		○			○			2	15	4.9	13.3	
		○		○				1	23	1.4	4.3	
		○	○					1	6	6.3	16.7	
	○						○	2	11	7.0	18.2	
	○					○		1	6	6.3	16.7	
	○				○			0	8		0.0	
	○			○				0	31		0.0	
	○		○					4	25	6.0	16.0	
	○	○						7	65	3.8	10.8	
3				○	○		○	0	1		0.0	11.5 (12/104)
			○	○			○	0	3		0.0	
		○			○		○	0	2		0.0	
		○		○		○		0	1		0.0	
		○	○		○			0	1		0.0	
		○	○	○				1	1		100.0	
	○				○	○		1	2	31.7	50.0	
	○			○			○	0	3		0.0	
	○			○		○		0	1		0.0	
	○			○	○			0	6		0.0	
	○		○				○	1	5	7.9	20.0	
	○		○		○			1	6	6.3	16.7	
	○		○	○				1	20	1.7	5.0	
	○	○					○	0	5		0.0	
	○	○				○		0	1		0.0	

Table 3 *(Continued)*

Number of AED	PHT	PB	PRM	CBZ	VPA	MPB	Other AED	Malformed	Total	O.R.	Incidence (%)	
	○	○			○			0	8		0.0	
	○	○		○				1	13	2.6	7.7	
	○	○	○					6	25	10.0	24.0	
4	○			○	○		○	2	4	31.7	50.0	13.5 (5/37)
	○			○	○	○		0	1		0.0	
	○		○	○		○	○	1	11	3.2	9.1	
	○		○	○		○		0	2		0.0	
	○		○	○	○			0	1		0.0	
	○	○		○			○	0	1		0.0	
	○	○	○				○	1	11	3.2	9.1	
	○	○	○		○			0	1		0.0	
	○	○	○	○				1	6	7.9	20.0	
5	○		○		○	○	○	1	2	31.7	50.0	15.4 (2/13)
	○		○	○		○	○	0	1		0.0	
	○		○	○	○		○	0	4		0.0	
	○	○		○	○	○		1	1		100.0	
	○	○	○		○		○	0	3		0.0	
	○	○	○	○			○	0	2		0.0	
Total (AED exposed)								80	885	3.1	9.0	

Table 4
Relationship between AED dose and occurrence of malformation in monopharmacy cases

Item	Category	Normal	Malformed	Total	Statistical test
PHT	$50 \leq D < 100$	8 (6.7)	0 (0.0)	8	
	$100 \leq D < 200$	20 (16.7)	1 (8.3)	21	
	$200 \leq D < 300$	55 (45.8)	5 (41.7)	60	
	$300 \leq D < 400$	32 (26.7)	5 (41.7)	37	
	$400 \leq D$	5 (4.2)	1 (8.3)	6	
	Maximum	450.000	400.000	450.000	Welch's test, $P = 0.220$
	Minimum	50.000	100.000	50.000	
	Median	200.000	250.000	200.000	
	Mean	226.700	262.083	229.917	
	S.D.	85.549	91.091	86.309	
PB	$D < 50$	5 (6.7)	1 (25.0)	6	
	$50 \leq D < 100$	13 (17.3)	1 (25.0)	14	
	$100 \leq D < 150$	26 (34.7)	2 (50.0)	28	
	$150 \leq D < 300$	29 (38.6)	0 (0.0)	29	
	$300 \leq D$	2 (2.7)	0 (0.0)	2	
	Maximum	300.000	125.000	300.000	Welch's test, $P = 0.120$
	Minimum	30.000	32.000	30.000	
	Median	100.000	75.000	100.000	
	Mean	125.467	76.750	123.000	
	S.D.	58.328	43.154	58.437	
PRM	$D < 400$	8 (26.7)	0 (0.0)	8	
	$400 \leq D < 600$	5 (16.7)	3 (60.0)	8	
	$600 \leq D < 800$	2 (6.7)	1 (20.0)	3	
	$800 \leq D < 1000$	0 (0.0)	0 (0.0)	0	
	$1000 < D$	15 (50.0)	1 (20.0)	16	
	Maximum	1500.000	1000.000	150.000	Welch's test, $P = 0.342$
	Minimum	100.000	400.000	10.000	
	Median	875.000	500.000	625.000	
	Mean	732.500	606.200	714.457	
	S.D.	376.357	233.489	359.498	
CBZ	$200 \leq D < 400$	24 (16.1)	1 (11.1)	25	
	$400 \leq D < 600$	21 (14.1)	2 (22.2)	23	
	$600 \leq D < 800$	43 (28.9)	2 (22.2)	45	
	$800 \leq D < 1000$	33 (22.1)	2 (22.2)	35	
	$1000 \leq D$	28 (18.8)	2 (22.2)	30	
	Maximum	1700.000	1600.000	1700.000	Welch's test, $P = 0.673$
	Minimum	200.000	200.000	200.000	
	Median	600.000	700.000	600.000	
	Mean	689.154	755.556	692.937	
	S.D.	344.089	447.524	349.362	
VPA	$D < 600$	19 (26.4)	0 (0.0)	19	
	$600 \leq D < 800$	18 (25.0)	1 (11.1)	19	
	$800 \leq D < 1000$	18 (22.2)	0 (0.0)	16	
	$1000 \leq D$	19 (26.4)	8 (88.9)	27	
	Maximum	2000.000	1600.000	2000.000	Welch's test, $P = 0.004$
	Minimum	200.000	600.000	200.000	
	Median	750.000	1200.000	800.000	
	Mean	774.486	1211.111	823.000	
	S.D.	405.788	337.062	420.195	

Table 4
Relationship between AED dose and occurrence of malformation in monopharmacy cases

Item	Category	Normal	Malformed	Total	Statistical test
Drug score	D<2	35 (7.9)	2 (5.6)	37	
	2≤D<4	104 (23.6)	4 (11.1)	108	
	4≤D<6	107 (24.3)	10 (27.8)	117	
	6≤D<8	99 (22.4)	9 (25.0)	108	
	8≤D<10	54 (12.2)	3 (8.3)	57	
	10≤D	42 (9.5)	8 (22.2)	50	
	Unknown	20	3	23	
	Maximum	20.000	16.000	20.000	Welch's test, $P = 0.040$
	Minimum	0.100	0.640	0.100	
	Median	5.000	6.000	5.000	
	Mean	5.578	7.086	5.696	
	S.D.	3.463	4.323	3.555	

D, dose.

given CBZ had received 200 mg or more. Among PHT or VPA exposed offspring with malformations, almost 90% of their mothers had received 200 mg or more of PHT and 1000 mg or more of VPA, respectively. The cut-off values of each AED dose for malformations determined by maximum chi-square test were 350 mg/day for PHT ($P = 0.157$, $n = 132$), 400 mg for PRM ($P = 0.512$, $n = 35$), 300 mg for CBZ ($P = 0.294$, $n = 158$), and 1000 mg for VPA ($P = 0.001$, $n = 81$). Only VPA dose reached a statistically significant level. There was not such a cut-off value for any AED dose in the polypharmacy group.

The cut-off values of AED level in the first trimester of pregnancy were 5 μg/ml for PHT ($P = 0.208$, $n = 83$), 3 μg/ml for CBZ ($P = 0.132$, $n = 106$), and 70 μg/ml for VPA ($P = 0.001$, $n = 59$). For PB and PRM, there was not such a value. Only VPA level was statistically significant. The cut-off value of the drug score was 13 in the polypharmacy group ($P = 0.095$, $n = 385$) as well as in all subjects treated with AED ($P = 0.001$, $n = 885$).

5. Discussion

Reported risk factors for congenital malformations in offspring vary from report to report (Dansky et al., 1982; Koch et al., 1982; Lindhout et al., 1982; Nakane, 1982; Kaneko et al., 1986, 1992). This is due mainly to the small number of subjects in each study which inevitably results in insufficient statistical power. Thus, this study was designed to collect many cases from epilepsy centers where collaborative study and precise follow-ups were possible.

A significantly higher incidence of malformations in the offspring of treated epileptic fathers as compared with those of untreated fathers as observed by Meyer (1973), suggests that there is either the existence of mutagenic effects of AEDs or differences in other uncontrolled risk factors between these two groups. A possible genetic factor was suggested by Dansky et al. (1982) and Greenberg et al. (1977), who reported an increased rate of malformation in the families of malformed children. However, these findings were not confirmed by other studies (Fraser et al., 1978; Annegers and Hauser, 1982).

The overall incidence of malformations in the AED treated group was 8.4% in the present study. It was 3.1% in AED unexposed infants, and 9.0% in the AED exposed group, which is consistent with reports that the incidence of malformations in offspring is two to three times higher than that in the general population (Kaneko, 1991). This increased incidence is believed to be attributable to AED rather than to epilepsy (Jager-Roman et al., 1986; Kaneko et al., 1988, 1992). The involvement of other risk factors, however, such as maternal hypoxia due to epileptic seizures

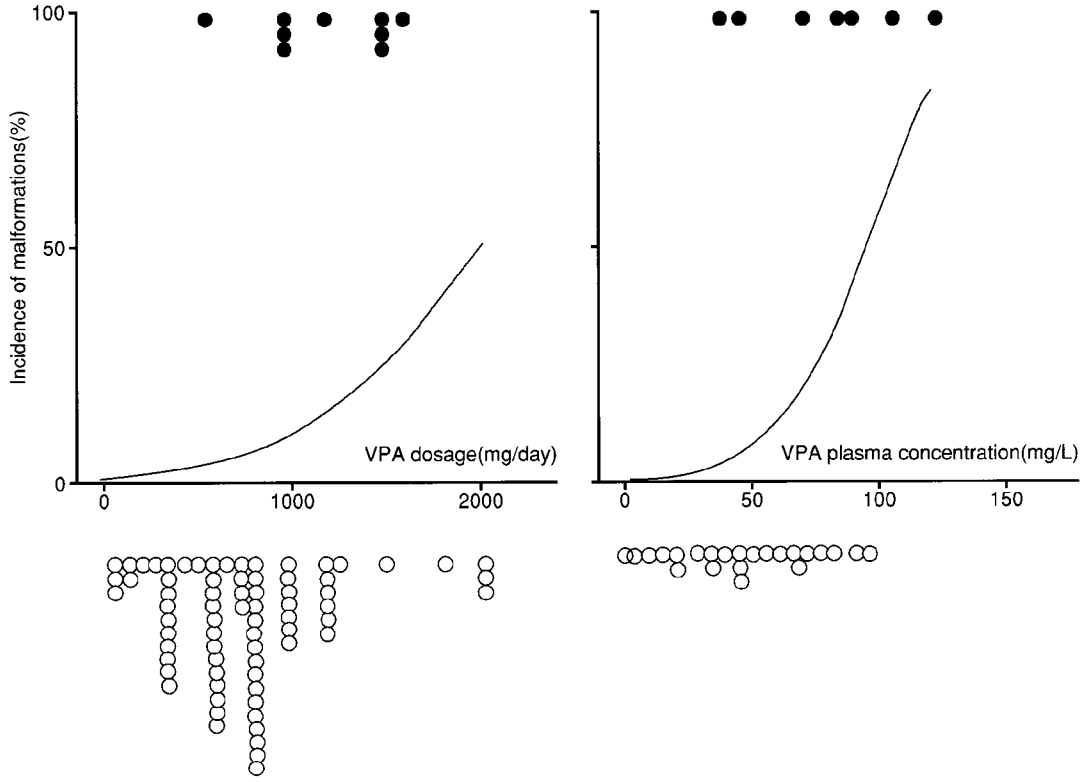

Fig. 1. Stimulus (VPA dose or level)-response (malformation) curves in VPA monopharmacy cases. (a) (Left panel) Stimulus (dose in mg/day)-response (malformation) curve (regression coefficient = 0.0021, $P = 0.0075$). (b) (Right panel) Stimulus (level in μg/ml)-response (malformation) curve (regression coefficient = 0.052, $P = 0.005$). Circles shown on the top (closed) and the bottom (open) of figures indicate mothers of infants with and those without malformations, respectively.

(Millicovsky, 1981), and genetic influences (Gaily et al., 1988) particularly for minor anomalies, must also be considered.

Various kinds of malformations were observed in the present study. The combined use of classical AEDs such as PB, PRM and PHT has been associated with heart defects and cleftings (Dansky et al., 1982; Lindhout et al., 1982). The more recently introduced VPA has been reported to be associated with spina bifida (Robert and Rosa, 1983; Lindhout and Schmidt, 1986). However, Rosa (1991) and Kaneko et al. (1993) reported a similar incidence of spina bifida in CBZ exposed offspring. Besides, despite the fact that the present study examined 983 infants including 500 cases with monopharmacy, this study showed significant association only between PRM exposure and inguinal hernia. Thus, the question whether or not there is a drug specific malformation remains unanswered.

No difference was found between drugs in the incidence of malformations in offspring exposed to a single AED, despite the fact that PRM exposure resulted in a three times higher incidence than PB exposure. However, incidences of malformations in offspring exposed to PRM or VPA were higher than that in offspring without drug exposure.

There was a cut-off value in VPA dose (1000 mg/day) and level (70 μg/ml) for the occurrence of malformations in offspring exposed to a single AED while there was no such value in the remaining AEDs in the monopharmacy as well as the polypharmacy groups. This suggests that there is

a significant contribution of toxic metabolites of AEDs which is evidently influenced by drug combination profiles. With regard to VPA dose and level or the DS (in polypharmacy cases), these cut-off values can be a good clinical guideline for AED treatment of women with epilepsy of childbearing age.

Regarding the etiology of birth defects, in the present study, although idiopathic epilepsy of the mothers showed a trend toward an increased incidence of malformations in offspring, none of the maternal risk factors studied, specifically maternal epileptic seizures with or without generalized convulsions during the first trimester of pregnancy, incidence of seizures during pregnancy, seizure type and classification of epilepsy showed a significant association with the occurrence of birth defects. Thus, the present study opposes the hypothesis that convulsive seizures of the mother leading to fetal hypoxia (Millicovsky, 1981) or any seizure type (Kaneko et al., 1984) is relevant to the increased malformations in offspring, and that the epileptic genotype itself does not predispose offspring to congenital malformations (Fraser et al., 1978). Therefore, it appears that

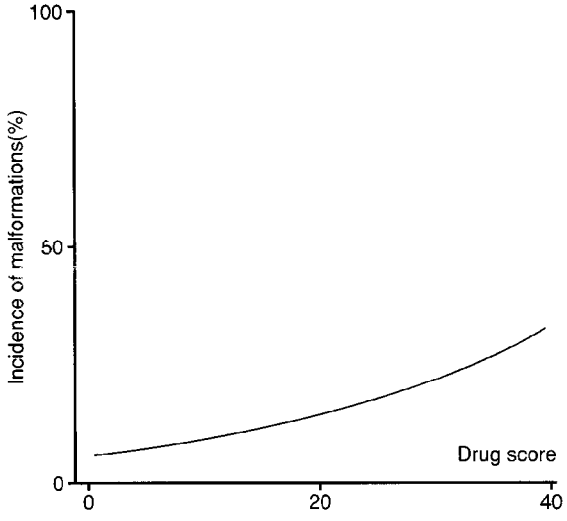

Fig. 2. Stimulus (drug score) and response (malformation) curve in all drug exposed cases. The ordinate indicates incidence of malformations (%), and the abscissa shows the drug score (regression coefficient = 0.0505, $P = 0.0059$).

AEDs rather than other factors confer a greater potential risk as teratogens. Among AED related factors, VPA dose, PHT dose, VPA level, the number of AEDs, and the DS were associated with malformations. The dissociation of PHT levels from the occurrence of malformations in spite of the significant association of PHT dose with birth defects may be due to a non-linear relationship between PHT dose and concentration which is described in the context of Michaelis-Menten kinetics (Kaneko et al., 1985).

Logistic regression analysis of the monopharmacy group as well as the AED treated group also revealed that, in both groups, VPA dose, VPA level, and the DS (not in monopharmacy group) were related to the occurrence of malformations. The incidence of malformations increased as the number of AEDs increased. Some AED combinations such as CBZ + VPA and PHT + PRM + PB showed particularly high rates of malformation. PHT, PRM, PB, MPB, and CBZ form chemically reactive oxidative (arene oxide) metabolites which covalently bind to embryonic macromolecules, thereby disrupting normal developmental processes (Martz et al., 1977; Winn and Wells, 1995). It has been proposed that polytherapy including AEDs that induce the cytochrome P450 system and/or inhibit the detoxification enzyme epoxide hydrolase increases fetal exposure to the toxic arene oxides (Buehler et al., 1990; Finnell et al., 1994). The human fetus is equipped with a hepatic monooxygenase system (Yaffe et al., 1979), which catalyzes the formation of epoxides (Pelkonen and Karki, 1975; Pacifici and Rane, 1983) and is also equipped with genetically determined (Buehler et al., 1990) hepatic and extrahepatic enzymes that metabolize the epoxides (Pacifici and Rane, 1983). VPA inhibits degradation of epoxide intermediates (Rambec et al., 1980), and also has inhibitory effects on glutathione *S*-transferases, another important enzyme which catalyzes the conjugation of glutathione with various electrophilic compounds (Tokinaga et al., 1996). On the other hand, an animal study clearly showed that high VPA levels produced a high incidence of birth defects (Nau et al., 1981). In humans, a high dose of VPA results in a high level of VPA as well as 4-en VPA

(Kondo et al., 1992), which is the most toxic metabolite of VPA (Nau and Losher, 1984). These metabolic interactions might explain the unusually high incidence of birth defects associated with such combinations as CBZ + VPA (Lindhout et al., 1984; Kaneko et al., 1988) and PHT + PRM + PB as observed in the present study. As background factors, only the presence of sibling's malformation related to the occurrence of malformations in the present study, which confirms Italian data (Battino et al., 1992). This might result from a genetically different drug metabolizing capacity in each offspring, such as epoxide hydrolase (Buehler et al., 1990) and glutatione *S*-transferase activities (Tokinaga et al., 1996). If the prediction of the genetically determined drug detoxifying capacity in each fetus is possible, clinical management of epileptic women of childbearing age may be significantly improved.

In conclusion, the increased incidence of congenital malformations is primarily caused by AEDs, which, in turn, suggests that malformations can be prevented by such measures as changing from polypharmacy to monopharmacy, and minimizing AED dose. In particular, a high level of VPA (more than 70 μg/ml) should be avoided, and in case of VPA administration slow release form of VPA (VPA retard) or three to four equally divided doses is recommended.

Among AED related factors, VPA dose, VPA level, the number of AEDs, and the DS were associated with malformations, and there was a possibility of contribution by toxic metabolites of AEDs influenced by drug combination profiles.

Regarding the etiology of birth defects, none of the maternal risk factors studied, specifically maternal epileptic seizures with or without generalized convulsions during the first trimester of pregnancy, incidence of seizures during pregnancy, seizure type and classification of epilepsy, showed a significant association with the occurrence of birth defects. However, further study is needed to confirm the possible relationship between idiopathic epilepsy in the mother and the increased incidence of malformations in offspring, and the question of whether or not there is a drug specific malformation also remains unanswered.

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